Chemical Composition and Antifungal Activity of Essential Oil from *Xanthium strumarium* L. Leaves

Z. PARVEEN*, S. MAZHAR, S. SIDDIQUE, A. MANZOOR[1] AND Z. ALI

PCSIR Laboratories Complex, Ferozpur Road, Lahore-54600, [1]Institue of Biochemistry and Biotechnology, University of the Punjab, Pakistan

Parveen, *et al.*: Studies on Essential Oil of *Xanthium strumarium* L. Leaves

The hydrodistilled essential oil from *Xanthium strumarium* L. leaves was analysed by gas chromatography-mass spectrometry. Nine out of twenty two constituents were identified from *X. strumarium* oil. The main components of the oil were β-caryophyllene (17.53%), α-cadinol (6.66%), spathulenol (6.09%), limonene (5.66%) and 1,3,5-trimethyl-2[2-nitroallyl]benzene (3.29%). Phytol (2.42%), α-muurolene (2.08%), copaene (1.47%) were present in appreciable amounts. E,E,Z-1,3,12-nonadecatriene-5,14-diol (0.27%) was present in minor amount. The oil displayed high degree of antifungal effect against all fungal strains with 11.8-46.0 mm zone of inhibition at concentration range 8-250 µg/ml. The 8 µg/ml minimum fungicidal concentration along with being the minimum inhibitory concentration points to the potential of *X. strumarium* essential oil as a promising source of antifungal agents with useful biomedical applications.

Key words: *Xanthium strumarium* L., antifungal activity, essential oil, gas chromatography/mass spectrometry, leaves

Fungal infections account for a high proportion of health problems in developing countries. There are alarming reports of opportunistic fungal infections particularly those of the skin and mucosal surface[1,2]. There is

***Address for correspondence**
E-mail: zahidaparveen18@gmail.com

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an increasing awareness amongst microbiologists and dermatologists pertaining to know the cause of infectious diseases in human being. Consequently, opportunistic fungi that were earlier reported from various plants as pathogens are now included under new spectrum of fungal pathogens.

The indiscriminate use of commercial antifungal drugs to treat infectious diseases coupled with resistance of microorganisms against antibiotics has forced the researchers to search for new antimicrobial substances from various sources including medicinal plants[3]. Current survey of literature reveals a gradual revival in the use of medicinal and aromatic plants in developed as well as in developing countries. There are 2600 plant species of which more than 700 find use as medicinal herbs[4]. Scientific research based on traditional use of medicinal plants indicated that plant-derived essential oils contain active antifungal substances, which could be safe and without side effects for treatment of specific fungal infections[5].

Cocklebur (*Xanthium strumarium* L.), an annual herb with stout, short and hairy stem, is mainly distributed in China and Europe. In traditional medicine, *X. strumarium* has been used for urticaria, headache, sinusitis, arthritis and emphysema[6-8]. All parts of plant possess sedative, diaphoretic and diuretic properties. The plant also shows its efficacy in mitigating long standing cases of malarial fever[9]. The genus *Xanthium* also reported to exhibit antibacterial[10], antiviral[11], antimalarial[12], fungicidal[13], insecticidal[14] and cytotoxic activities against cancer cell lines[15]. Commercially this plant is used in yellow dye manufacturing[16].

The chemical composition of *X. strumarium* has been found to include phenolic compounds as thiazolidinediones, chlorogenic acids, ferulic acids[6], 1,3,5-tri-O-caffeoyl quinic acid, 1,5-di-O-caffeoyl quinic acid, caffeic acid[17], as well as isoprenoids such as strumasterol, β-sitosterol[18], triterpenoid saponins[18], monoterpene, sesquiterpene hydrocarbons[19] and xanthanolide sesquiterpene lactones[20]. However, there are few reports documented about the antifungal and fungicidal activity of the oil of *X. strumarium*[13], especially very limited data is available about the locally growing *X. strumarium* Pakistani plant. Therefore, the aim of this work was to analyse chemical composition and to determine antifungal activity of hydrodistilled oil of *X. strumarium* leaves to generate comparable data from Pakistan.

Fresh leaves of *X. strumarium* were collected from the PCSIR Labs Complex, Lahore, Pakistan and authenticated at the Herbarium, Department of Botany, University of Punjab, Lahore, Pakistan. A voucher specimen (code 15081) was also deposited in the herbarium. Anhydrous sodium sulphate, ethanol and methanol used in this study were purchased from Merck (Darmstadt, Germany). Culture media, potato dextrose agar (PDA) and yeast extract peptone dextrose (YPD) broth were purchased from Oxoid Limited, Hampshire, UK.

Air-dried and finely ground plant material was subjected to hydro distillation for 6 h using Reverse Dean-Stark apparatus[21]. Distillate of essential oil was dried over anhydrous sodium sulphate, filtered and stored at –4° until analysed. The analysis of the essential oil was carried out using gas chromatography/mass spectrometry (GC/MS) of Agilent Technologies Inc., USA, Model 6890 N, operating in electron ionization mode at 70 eV equipped with a split less injector. Helium is used as a carrier gas at the flow rate of 1 ml/min using DB-5 MS (30×0.25 mm id, 0.25 µ film thickness) capillary column. The initial temperature was programmed at 50-140° at the rate of 5°/min and then 140-250° at the rate of 3°/min followed by a constant temperature at 260° for period of 20 min. Sample (2 µl) was injected to the column programmed at 200° and resolutions of components were attained. The mass spectrometer is capable of scanning from 35 to 500 atomic mass unit (AMU) every second or less. The data acquisition system continuously acquires and stores all data analyses.

The identification of chemical components was based on the comparison of their mass spectra with those of NIST mass spectral library[22,23] and those described by Adam[24] as well as on comparison of their retention indices (RI) either with those of authentic compounds or with literature values[24].

Six fungal strains with marked Accession No (AN) were purchased from fungal bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan to demonstrate antifungal activity of essential oil. All selected fungal strains, *Aspergillus niger* (AN1109), *A. flavus* (AN1110), *Fusarium oxysporum* (AN1175), *F. solani* (AN1199), *Alternaria alternata* (AN1200) and *Penicillium digitatum* (AN1160) were sub-cultured at 25° for 120 h on PDA slants to prepare spore suspension before testing antifungal activity.

Antifungal activity of six different concentrations

of *X. strumarium* essential oil was evaluated by agar well diffusion method on PDA against selected fungal strains[25]. Twenty ml of molten agar medium was inoculated with spore suspension of indicator fungal strain at 10^6 CFU/ml. The inoculated medium was poured into a petri plate and allowed to solidify. Wells were made on solidified agar and 90 µl of the respective concentration of essential oil was added into well to evaluate antifungal and minimum inhibitory concentration (MIC). The plates were incubated at 25° for 48 h. The diameters of zone of inhibition zones were measured in millimetres and results were recorded in triplicate. MIC was defined as lowest concentration of essential oil to inhibit growth of fungal strains after incubation.

Minimal fungicidal concentration (MFC) was determined by broth dilution method[26]. Tubes containing culture broth and oil with concentration of MIC were inoculated with 10^6 CFU/ml fungal spores. Tubes with and without respective fungal spore load were used as controls. The tubes were incubated at 25° for 48 h to examine any visible turbidity. After incubation, 100 µl from tubes showing no visible growth was removed and poured into plates along with agar to calculate total emergent mold counts. The minimum oil concentration that entirely inhibits the fungal growth, killing 99.9% of the original inoculum after 48 h of incubation was considered as MFC.

The yield of oil obtained by hydrodistillation was 0.13% (v/w), in agreement with Taher *et al.*[19] who reported a yield of 0.12%. The gas chromatography coupled with mass spectrometry revealed the presence of twenty two compounds. Nine components, 45.20% of total were identified (Table 1). These components were further classified in two fractions i.e. hydrocarbon fraction and oxygenated fraction. Hydrocarbon fraction constituted β-caryophyllene, limonene, α-muurolene, copaene and while α-cadinol, spathulenol, phytol, 1,3,5-trimethyl-2[2-nitroallyl]benzene and E,E,Z-1,3,12-nonadecatriene-5,14-diol comprised the oxygenated fraction of the oil.

TABLE 1: GC/MS ANALYSIS OF ESSENTIAL OIL OF *X. STRUMARIUM* LEAVES

Name of components	RI	Relative % In essential oil
Limonene	1032	5.66
Copaene	1377	1.47
ß-Caryophyllene	1418	17.53
Spathulenol	1572	6.09
α-cadinol	1652	6.66
1,3,5-trimethyl-2[2-nitroallyl]benzene		3.29
α-Muurolene	1499	2.08
Phytol	1821	2.42
E,E,Z-1,3,12-nonadecatriene-5,14-diol		0.27

In the present study, the major component of the essential oil was found to be β-caryophyllene (17.53%), which did not agree with the previous results in which β-guaiene[27,28] and limonene[29-31] were reported to be the major components. It is interesting to note that β-guaiene, which had been reported as major constituent of leaves oil[27,28] was found to be absent in this study. Limonene (5.66%) was present in low concentration as compared to previous reports[27-31]. Phytol (2.42%) concentration was in agreement with an earlier report[28]. α-cadinol (6.66%), spathulenol (6.09%), 1,3,5-trimethyl-2[2nitroallyl] benzene (3.29%), α-muurolene (2.08%), copaene (1.47%) and E,E,Z-1,3,12-nonadecatriene-5,14-diol (0.27%), were reported first time from the essential oil of *X. strumarium.*

The antifungal activity of *X. strumarium* essential oil was evaluated by measuring the zone of inhibition against six fungal strains. All assayed concentrations (8, 25, 65, 100, 150 and 250 µg/ml) of oil exhibited remarkable antifungal activity against the tested fungal strains with zones of inhibition ranging from 11.0 to 39.0 mm (Table 2). However, the degree of inhibition was dependent on the oil concentrations and the fungal strains. Maximum antifungal activity was exhibited against *F. solani* (16.1-39.0 mm) followed by *P. digitatum* (11.0-38.1 mm) and *A. niger* (11.2-35.7 mm) at 8-250 µg/ml concentrations. The high degree of antifungal activity was further confirmed by MIC and MFC. The observed very low value of MIC as well as MFC i.e. 8 µg/ml revealed that all fungal strains were highly sensitive to *X. strumarium* essential oil (Table 3). According to Duarte *et al.*[32] proposed plant material classification, based on the MIC results, the oil or plant be regarded as strong inhibitors when MIC is below 0.5 mg/ml, moderate when MIC is in range of 0.6-1.5 mg/ml and weak when MIC is above 1.6 mg/ml. Considering the plant classification for potential antimicrobial activity reported by Duarte *et al.*[33], the *X. strumarium* oil with antifungal activity at 8-250 µg/ml concentration against the evaluated fungal strains falls within the group of strong inhibitor plants. These remarkable antifungal and fungicidal results are in agreement with those reported in previous studies[27,33-36], notwithstanding the differences in

TABLE 2: ANTIFUNGAL ACTIVITY OF *X. STRUMARIUM* ESSENTIAL OIL BY AGAR WELL DIFFUSION METHOD

Tested fungal strains	Zone of inhibition (mm)* (fungistatic effect)					
	250 µg/ml	150 µg/ml	100 µg/ml	65 µg/ml	25 µg/ml	8 µg/ml
A. niger	35.7±0.2	25.3±0.3	20.1±0.4	14.8±0.1	12.7±0.2	11.2±0.1
A. flavus	34.5±0.3	24.1±0.3	19.8±0.7	14.2±0.1	12.5±0.3	11.3±0.1
F. oxysporum	31.8±0.5	23.5±0.4	18.3±0.1	15.8±0.5	12.8±0.2	11.2±0.1
F. solani	39.0±0.0	28.5±0.8	24.8±0.2	21.5±0.3	18.8±0.2	16.1±0.1
A. alternata	30.2±0.3	26.9±0.5	22.5±0.4	19.5±0.4	16.3±0.3	15.0±0.0
P. digitatum	38.1±0.2	27.1±0.7	18.8±0.0	14.8±0.4	12.2±0.0	11.0±0.0

*The diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as mean±SD of triplicate experiments

TABLE 3: FUNGICIDAL CONCENTRATION (MFC) OF *X. STRUMARIUM* ESSENTIAL OIL

Tested fungal strains	MIC (µg/ml)	MFC (µg/ml)
A. niger	8	8
A. flavus	8	8
F. oxysporum	8	8
F. solani	8	8
A. alternate	8	8
P. digitatum	8	8

selected fungal strains and the method used to evaluate antifungal activity.

Phytopharmacological research approach on the biological activity possessed by the essential oil has disclosed that chemical composition of the essential oil could be linked with the antimicrobial activity and the reported therapeutic effects. The sesquiterpene β-caryophyllene, the most abundant constituents found in our essential oil was extensively investigated because of its several biological activities, including antimicrobial[37,38], insecticidal[39,40], antiinflammatory[41,42], anticarcinogenic[43-47] and local anesthetic[48] activities. Limonene present in appreciable amounts (5.66%) in our oil efficient in inhibiting the proliferation of a variety of microorganisms that cause food spoilage[49]. The antimicrobial activity of phytol against eight bacterial and eight fungal strains was investigated by Pejin *et al*.[50]. It was proven that phytol to be active against all tested bacteria and fungi. According to Nada *et al*.[51] and Tabanca *et al.*[52] spathulenol, demonstrated significant antimicrobial activity. Ho *et al.*[53] demonstrated that α-cadinol has antifungal activity. 1,3,5-trimethyl-2[2nitroallyl] benzene, α- muurolene, copaene and E,E,Z-1,3,12-nonadecatriene-5,14-diol present in our essential oil but there is no data available on their antimicrobial activity. From the results obtained in this investigation, it can be concluded that antifungal activity of the essential oil of *X. strumarium* leaves could be attributed to the presence of sesquiterpenes and oxygenated terpenes. The strong antifungal activity might be due to a possible synergetic effect of compounds, which were identified for the first time in this essential oil.

Reported results of this study revealed that the essential oil of *X. strumarium* possessed antifungal activity against a wide spectrum of fungal strains. Remarkable growth inhibition of tested fungal strains suggested that *X. strumarium* could serve as a starting point for finding selective antifungal agents, which could have potential clinical applications in the treatment of fungal infectious diseases.

Conflict of interests:

Authors report no conflict of interests.

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